OsteoArthritis and Cartilage (2007) **15,** 226–231

doi:10.1016/j.joca.2006.08.008

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Brief report
Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells[1]

R. Kuroda M.D.†*, K. Ishida M.D.†, T. Matsumoto M.D.†, T. Akisue M.D.†, H. Fujioka M.D.†, K. Mizuno M.D.†, H. Ohgushi M.D.‡, S. Wakitani M.D.§ and M. Kurosaka M.D.†

† *Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Japan*

‡ *Research Institute for Cell Engineering (RICE), National Institute of Advanced Industrial Science and Technology (AIST), Japan*

§ *Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, Japan*

Summary

Objectives: Human bone-marrow stromal cells are believed to be multipotent even in adults. This study assessed the effectiveness of autologous bone-marrow stromal cells, which were embedded within a collagen scaffold, to repair a full-thickness articular cartilage defect in the medial femoral condyle of an athlete.

Patient and methods: A 31-year-old male judo player suffering from pain in the right knee was reviewed. A 20 × 30-mm full-thickness cartilage defect (International Cartilage Repair Society classification (ICRS) grade IV) was revealed in the weight-bearing area of the medial femoral condyle. With the informed consent of the patient, the defect was treated with autologous bone-marrow stromal cells. Bone marrow was aspirated from the iliac crest of the patient 4 weeks before surgery. After removing the erythrocytes, the remaining cells were expanded in culture. Adherent cells were collected and embedded within a collagen gel, which was transferred to the articular cartilage defect in the medial femoral condyle. The implant was covered with an autologous periosteal flap.

Results: Seven months after surgery, arthroscopy revealed the defect to be covered with smooth tissues. Histologically, the defect was filled with a hyaline-like type of cartilage tissue which stained positively with Safranin-O. One year after surgery, the clinical symptoms had improved significantly. The patient had reattained his previous activity level and experienced neither pain nor other complications.

Conclusions: Our findings indicate that the transplantation of autologous bone-marrow stromal cells can promote the repair of large focal articular cartilage defects in young, active patients.

Key words: Bone-marrow stromal cell, Autologous cell implantation, Athlete, Cartilage repair, Hyaline cartilage.

Introduction

Articular cartilage defects have a poor capacity to undergo self-repair, owing to the low mitotic potential of chondrocytes *in vivo*[1]. Since articular cartilage defects can progress to osteoarthritis in some patients, they need to be repaired even though their exact natural course remains obscure[2–4]. Strategies that have been instigated to repair articular cartilage defects include microfractuing[5] and mosaicplasty[6,7]. However, these procedures are limited to small- and medium-sized focal chondral and osteochondral defects. Mosaicplasty is also limited by the need to create defects at donor sites, by an insufficient repair result between the grafts, and by the technical difficulties experienced in resurfacing the original curvature of the joint[6,7]. The autologous chondrocyte implantation (ACI) technique was first performed by Peterson *et al.*[8] in 1994. This was the first application of a cell-engineering strategy in orthopaedic surgery. However, several problems are associated with the procedure. These include difficulties in obtaining a sufficient number of chondrocytes for autotransplantation, the necessity of creating donor-site defects within autologous cartilage, and poor histological repair[1,6].

Mesenchymal stromal cells within the adult bone marrow are multipotent, being capable of forming bone, cartilage and other connective tissues[9]. It has been suggested that these cells may be used effectively for the repair of cartilage tissue. We report on a case of a focal articular cartilage injury in an athlete. This study assessed the effectiveness of autologous bone-marrow stromal cells to repair a full-thickness articular cartilage defect in the medial femoral condyle of a 31-year-old male judo player.

Case report

In 1999, a 31-year-old male judo player injured his right knee whilst playing judo, and he underwent medial meniscectomy at another hospital. He resumed his judo activities

[1]No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

*Address correspondence and reprint requests to: Ryosuke Kuroda, M.D., Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Tel: 81-78-382-5987; Fax: 81-78-351-6944; E-mail: kurodar@med.kobe-u.ac.jp

Received 11 May 2006; revision accepted 12 August 2006.

and experienced no pain. However, in May 2004, he twisted his knee whilst playing judo. He complained of pain in the right knee and was referred to our hospital. His right knee was remarkably swollen, and the active range of motion was limited to extension. There were no signs of collateral or cruciate ligament instability. X-radiography of the weight-bearing knee in the standing position disclosed a radiolucent area in the medial femoral condyle [Fig. 1(A)], but the limb alignment was normal. Magnetic resonance imaging (MRI) revealed a cartilage defect within the medial femoral condyle, oedema of the subchondral bone, and degeneration of the posterior part of the medial meniscus [Fig. 2(A, B)]. Laboratory tests disclosed no abnormalities.

Arthroscopy revealed a 20×30-mm full-thickness cartilage defect (International Cartilage Repair Society classification (ICRS) grade IV) within the weight-bearing area of the medial femoral condyle [Fig. 3(A)] and a macroscopic tear in the middle third of the medial meniscus. A partial meniscectomy was performed, but the cartilage defect was left untreated. In an attempt to repair the articular cartilage defect, we decided to perform an autologous transplantation of bone-marrow cells, with the informed consent of the patient. Fifteen millilitres of bone marrow were aspirated from the left iliac crest and transferred to tubes containing heparinized phosphate-buffered saline. Four hundred millilitres of the patient's blood were collected. After centrifugation, 180 ml of serum were obtained. The cells derived from 3 ml of fresh bone marrow were transferred to two 75-cm^2 plastic culture flasks, within which they were maintained for 4 weeks, with changes of medium (alpha minimal essential medium supplemented with 15% autologous serum) three times per week. At the time when the medium was changed, non-adherent haematopoietic cells were removed. After about 10 days, the number of adherent cells had increased to several million. The cells were collected after trypsinization (first passage) and further cultured (subcultured) in other flasks for about 10 days. At this stage, they had a fibroblast-like appearance. They were negative for markers of haematopoietic cells (CD14, CD34) and for HLA-DR, but positive for markers of mesenchymal ones (CD73, CD90, CD105). These findings indicate that the adherent cultured cells were of the mesenchymal type[10]. The subcultured cells were collected and suspended within a 1% acid-soluble solution of porcine tendon type-I collagen (final cell density: 5×10^6 cells/ml). The collagenous cell suspension was placed on a sheet of collagen (Gunze, Kyoto, Japan), which acted as a support, and gelled at 37°C. This gel–cell composite was further cultured for a couple of days. A small aliquot of medium collected at the time of the last medium change was used to check for bacterial and fungal contamination. These tests were negative. Hence, the composite was transplanted. The entire culturing procedure was conducted at the Cell Processing Center (CPC) of the National Institute of Advanced Industrial Science and Technology (AIST). The CPC is an ISO13485 certified facility.

Transplantation surgery was performed in September 2004. Following a medial para-patellar approach, all fibrous tissue covering the surface of the defect was removed. The subchondral bone was not stimulated. The gel–cell composite was introduced into the defect and covered with an autologous periosteal flap (harvested from the anterior surface of the tibia), with the cambial layer facing the bone marrow. The autologous periosteal patch was affixed to the surrounding rim of the normal cartilage with interrupted absorbable sutures. The knee was immobilised for 10 days with a knee brace. Continuous passive motion was initiated 11 days after surgery. Partial weight-bearing was instigated 4 weeks, and full weight-bearing 8 weeks after surgery.

Arthroscopy was performed 7 months after surgery. The defect was completely covered with smooth tissues [Fig. 3(B)], which appeared to have a firm consistency on probing. Histologically, the defect was filled with three distinct layers of repaired tissue. The first (superficial) layer consisted of fibrous tissue and was presumably the periosteal patch. The second (middle) layer was composed of a hyaline-like type of cartilage tissue, which stained positively with both Safranin-O and Toluidine Blue [Fig. 4(A, B)]. The third (lower) layer was subchondral bone. Imaging at higher magnification revealed cells within the middle layer to have a chondrocyte-like appearance [Fig. 4(C)]. Immunohistochemistry for type-II collagen revealed a positive reaction [Fig. 4(D)]. One year after surgery, X-radiography disclosed no radiolucent area

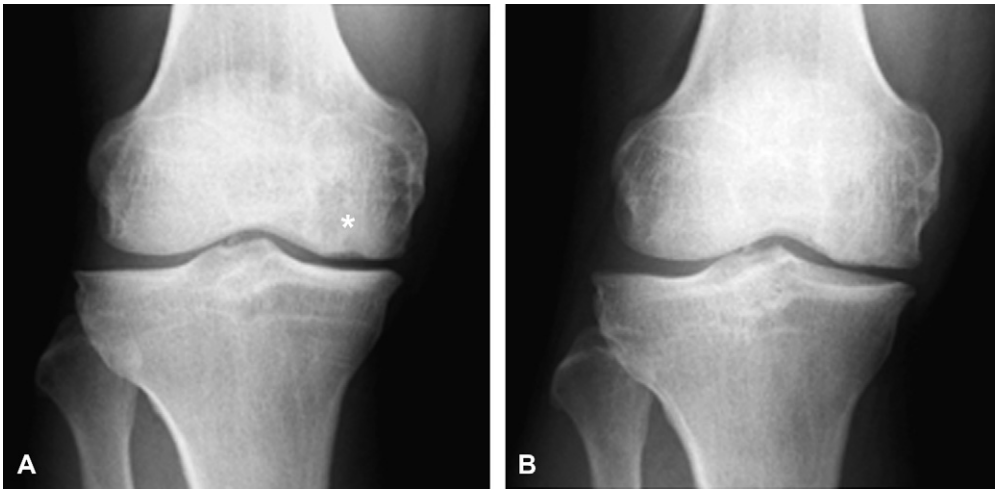

Fig. 1. (A) Anterior–posterior X-radiographic view of the weight-bearing knee before surgery. The image reveals a radiolucent area (*) within the medial femoral condyle. (B) Anterior–posterior X-radiographic view of the weight-bearing knee 1 year after surgery. No radiolucent area is now apparent within the medial femoral condyle.

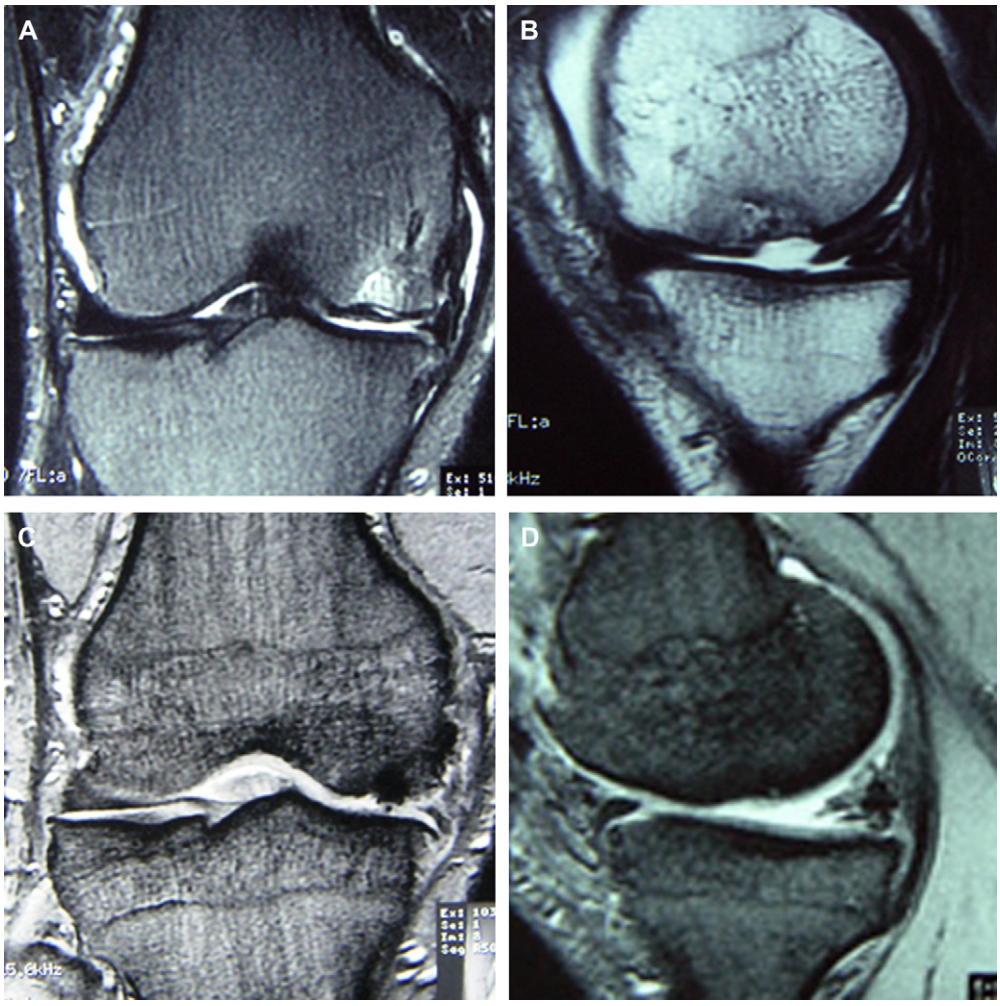

Fig. 2. (A, B) Magnetic resonance images before surgery. A defect is apparent within the medial femoral condyle, the subchondral bone is oedematous, and the posterior portion of the medial meniscus has degenerated. (C, D) Magnetic resonance images 1 year after surgery. The bone is no longer oedematous, but chondral and subchondral irregularities are apparent in the repaired area.

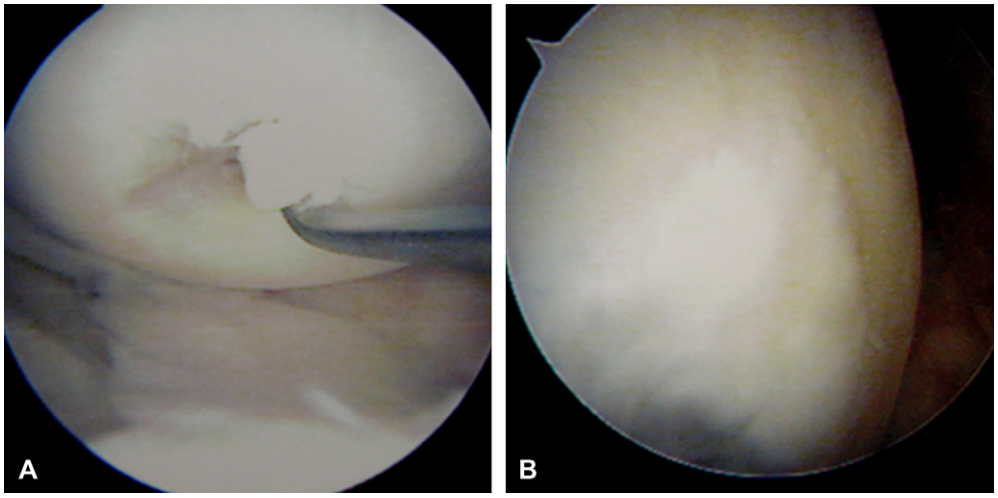

Fig. 3. (A) Arthroscopic findings before surgery. A 20 × 30-mm full-thickness cartilage defect (ICRS grade IV) is apparent within the weight-bearing area of the medial femoral condyle. (B) Arthroscopic findings 7 months after surgery. The defect is completely covered with tissue, which is a little softer than the surrounding articular cartilage.

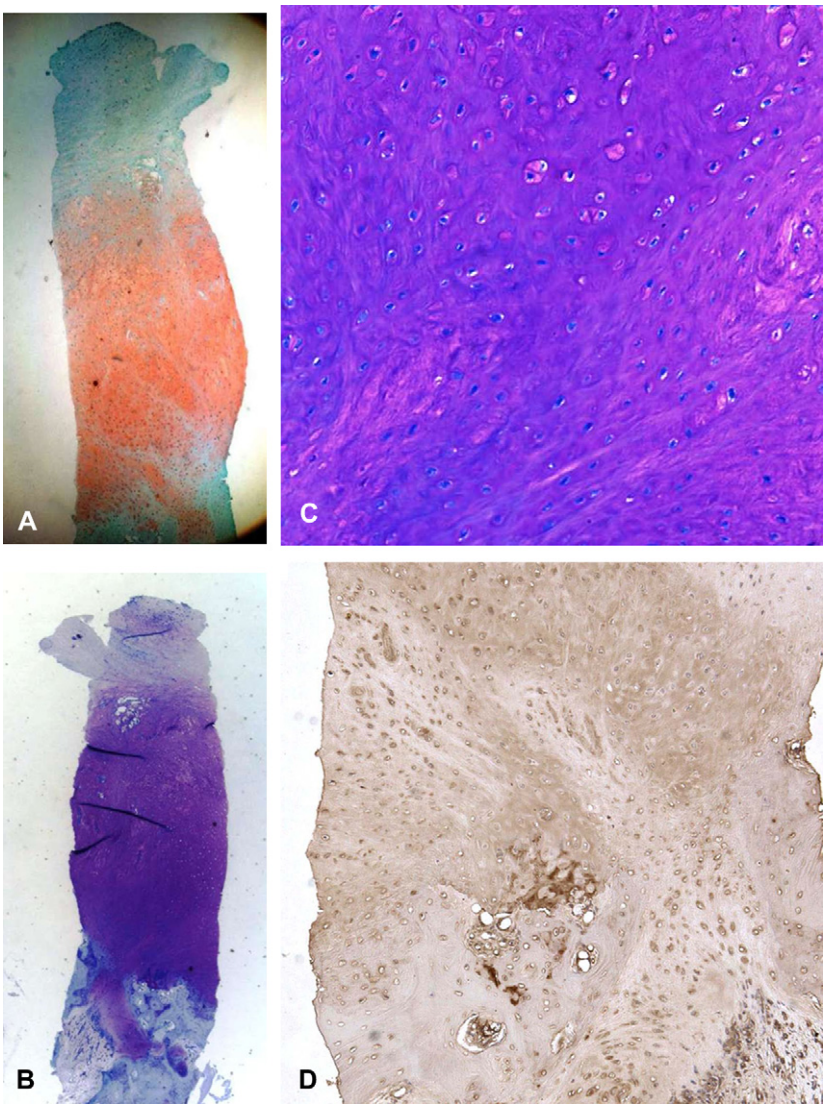

Fig. 4. High-resolution imaging of the defect area 1 year after surgery. (A) Appearance of the tissue after staining with Safranin-O. Three distinct layers are apparent. The first (upper) layer consists of fibrous tissue, and is presumably the periosteal patch. The second (middle) layer is composed of a hyaline-like type of cartilage tissue, which stains positively with Safranin-O. The third (lower) layer is subchondral bone. (B) After treatment with Toluidine Blue, the middle layer stains metachromatically. (C) Higher-magnification view of the Toluidine-Blue-stained middle layer of tissue revealing the chondrocyte-like appearance of the cells and an abundant extracellular matrix. (D) Immunohistochemical staining of the tissue for type-II collagen reveals a positive reaction within the middle layer.

[Fig. 1(B)]. However, MRI revealed focal chondral and subchondral irregularities within the repaired area [Fig. 2(C, D)]. Clinical symptoms had improved significantly. The patient had resumed his previous activity level and experienced neither pain nor other complications.

Discussion

Articular cartilage has a poor intrinsic capacity for repair[11]. Even a small defect caused by mechanical damage will fail to heal. Indeed, it undergoes further degeneration with time, often progressing to the debilitating condition of osteoarthritis[12,13].

Human bone-marrow stromal cells are believed to be multipotent, and even in adults they have the potential to differentiate into bone, cartilage, fat, tendon and muscle[9]. When these cells are implanted within such defects, they appear to be capable both of differentiating into articular cartilage and of forming subchondral bone.

Defects that are confined to the articular cartilage layer do not heal spontaneously. The usual inflammatory response is triggered, but an inferior, fibrocartilaginous type of tissue is formed. Techniques that have been instigated to treat arthritis and chondral damage include abrasion[14], bone-marrow stimulation[5], autografting, allografting and cell transplantation[1]. Recently, the ACI procedure was introduced[1], and has since been widely performed. However, the outcome of this surgical treatment strategy is still controversial. Although clinical symptoms improve, histological analyses have revealed the repaired tissue to be insufficient[1,6].

The transplantation of culture-expanded bone-marrow stromal cells has the advantage of not requiring an additional arthroscopic procedure to harvest articular cartilage, and of being suitable for large focal cartilage defects. This

procedure is clinically much easier to perform, since it involves no cell collection. The cells are collected under conditions of local anaesthesia at an outpatient clinic. Furthermore, neither bone nor cartilage defects are created during the collection of the autologous bone-marrow stromal cells, and these can proliferate without loosing their capacity to differentiate[15]. Wakitani *et al.*, who were the first to report good results after the transplantation of autologous bone-marrow stromal cells in an animal model[15], initiated the procedure in humans. The reported cases include the treatment of patellar cartilage defects[16] and of osteoarthritic knees undergoing high tibial osteotomy[17]. Histological analyses revealed the defects to be repaired with fibrocartilaginous tissue[16]. In the present study, we report for the first time on the implantation of autologous bone-marrow stromal cells within a focal cartilage defect of the weight-bearing area of the medial femoral condyle in an athlete. The patient has expressed his satisfaction with the outcome. Seven months after transplantation, arthroscopy revealed the defect to be completely filled with cartilaginous tissue and to be covered with a smooth surface. At no location was the subchondral bone exposed. The histological analysis revealed the defect to be repaired with a hyaline-like type of cartilage tissue which was rich in extracellular matrix. The bony compartment of the defect was also repaired. We presume that the transplanted bone-marrow stromal cells differentiated into both cartilage and bone. The result achieved is notably better than the outcome reported by other investigators who have applied the same technique in humans[16,17]. After ACI, the repair tissue formed in the patella has been reported to be substantially inferior to that laid down in the femoral condyle[1]. We consider that the reparative capacity of a chondral defect is linked to its topographic location. Appropriate mechanical conditions may be necessary to provide a suitable environment for the synthesis of an extracellular-matrix-rich cartilage[16]. Furthermore, it has been postulated that the formation of fibrocartilage may be inhibited by preventing bleeding from the subchondral bone. Most mesenchymal tissues derive their nutrients from a well-developed network of capillaries. But cartilage is normally devoid of capillary networks and, except during endochondral bone formation, is resistant to vascular invasion from surrounding tissues[18]. Since the vascular barrier is broken down in full-thickness cartilage defects, various cells and cytokines, including fibroblasts, haematopoietic stem cells and angiogenic growth factors, would invade the damaged cartilage if the subchondral bone were stimulated. In our case, the subchondral bone was not stimulated. Hence, the environment favoured cartilage regeneration. In previous reports, the tissue derived from implanted chondrocytes was not invaded by vessels or replaced by subchondral bone, and the repair cartilage maintained its thickness throughout the depth of the original defect[19,20]. These findings may also reflect an absence of bone stimulation.

The standard set for full healing is high, and has not yet been achieved. Promising approaches could involve the combination of bone-marrow stromal cells with various scaffolds and growth factors, using either recombinant proteins or the gene-therapy approach. Although further studies with a large number of patients and longer follow-up periods are required to investigate the long-term efficacy of this procedure, the conclusion of our study is that the transplantation of autologous bone-marrow stromal cells is an acceptable procedure to treat full-thickness focal chondral defects of critical size (2–10 cm^2) in young (15–50 years of age), active patients, including athletes.

References

1. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331:889–95.
2. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy 2002;18:730–4.
3. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cartilage 2002;10: 432–63.
4. Mankin HJ. The response of articular cartilage to mechanical injury. J Bone Joint Surg Am 1982;64:460–6.
5. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy 2003;19:477–84.
6. Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. J Bone Joint Surg Am 2003;85A:185–92.
7. Matsusue Y, Yamamuro T, Hama H. Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy 1993;9:318–21.
8. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. J Bone Joint Surg Am 2003;85A(Suppl 2):17–24.
9. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
10. Kotobuki N, Hirose M, Takakura Y, Ohgushi H. Cultured autologous human cells for hard tissue regeneration: preparation and characterization of mesenchymal stem cells from bone marrow. Artif Organs 2004;28: 33–9.
11. Buckwalter JA, Mankin HJ. Articular cartilage repair and transplantation. Arthritis Rheum 1998;41:1331–42.
12. Fuller JA, Ghadially FN. Ultrastructural observations on surgically produced partial-thickness defects in articular cartilage. Clin Orthop 1972;86:193–205.
13. Ghadially FN, Fuller JA, Kirkaldy-Willis WH. Ultrastructure of full-thickness defects in articular cartilage. Arch Pathol 1971;92:356–69.
14. Friedman MJ, Berasi CC, Fox JM, Del Pizzo W, Snyder SJ, Ferkel RD. Preliminary results with abrasion arthroplasty in the osteoarthritic knee. Clin Orthop 1984;182:200–5.
15. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, *et al*. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 1994;76:579–92.
16. Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. Cell Transplant 2004;13:595–600.
17. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 2002;10:199–206.

18. Shukunami C, Oshima Y, Hiraki Y. Chondromodulin-I and tenomodulin: a new class of tissue-specific angiogenesis inhibitors found in hypovascular connective tissues. Biochem Biophys Res Commun 2005;333:299–307.
19. Wakitani S, Goto T, Young RG, Mansour JM, Goldberg VM, Caplan AI. Repair of large full-thickness articular cartilage defects with allograft articular chondrocytes embedded in a collagen gel. Tissue Eng 1998;4:429–44.
20. Kawamura S, Wakitani S, Kimura T, Maeda A, Caplan AI, Shino K, *et al*. Articular cartilage repair. Rabbit experiments with a collagen gel-biomatrix and chondrocytes cultured in it. Acta Orthop Scand 1998; 69:56–62.